



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Suman Preet Singh Khanuja, et al.

Serial No.: 09/487,405

Group No.: 1634

Filed: January 18, 2000

Examiner.: A. K. Chakrabarti

For: NOVEL SCREENING METHOD FOR SELECTION OF INSECT  
TOLERANT PLANTS

Attorney Docket No.: U 012567-2

Assistant Commissioner of Patents  
Washington, D.C. 20231

SUPPLEMENTAL RESPONSE

Reconsideration and further examination is respectfully requested in  
view of the following remarks.

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## REMARKS

The Examiner is respectfully requested to consider the following information in support of the patentability of the claimed invention.

**An embodiment of the invention involves the following:**

1. Generation of large number of plantlets through for example tissue culture using developed specific medium leading to a large proportion of somaclonal variants



2. Screening regenerated plantlets for establishing variation at DNA level through RAPD to identify somaclonal variants.



3. Screening identified genetic variants (DNA variants) through forced feeding by insect larvae at for example the tissue culture stage itself.



4. Selection of non-fed tolerant plantlets.



5. Multiplication of the tolerant plants in for example tissue culture using specific medium developed for stable micropropagation.



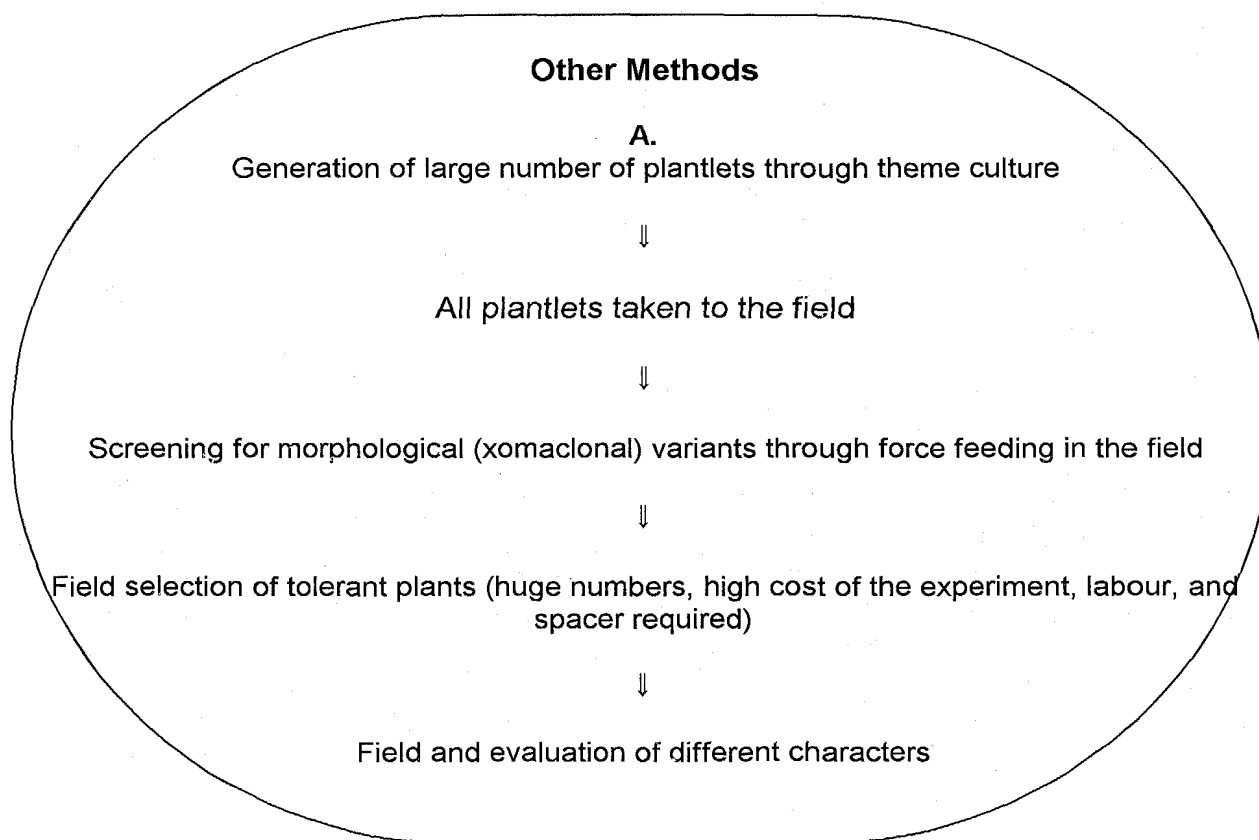
6. Transfer to the field and evaluation of different characteristics including insect tolerance.

**Novelty:**

1. No reference describes the steps 1-4 as set out above.
2. The sequence has not been suggested by any one else.
3. The method uses techniques invented earlier like RAPD and tissue culture but to achieve the goal of speedy screening for tolerance with reduced cost, labour and field area as compared to any other method.
4. Introduction of RAPD after tissue culture step will identify plants with DNA change indicating change in genotypes. Therefore, the total number of plants generated in tissue culture to be considered for screening are reduced drastically where as in conventional methods all the plants are screened, sometimes entering into a problem like reversion of characters. Reduction of the number of plants for screening reduces

the cost of the experiment, labour, and space when these are taken to the field directly.

5. Only screening of the plants confirmed for genetic change is required, which is possible in the small size tissue culture stage itself through larval forced feeding to further reduce the cost, labour, space and time compared to screening the huge number of plants in the field.
6. Only those plants with confirmed genetic change and tolerant to insect attack are taken to the field and therefore screening is not in the field but at culture level and in field it is evaluation of selected clones only.



Disadvantage:

1. Plants can not be selected as somaclones on the basis of morphology alone. Earlier workers have reported through morphological selection the plants may revert back after a few generations as morphological characters are dependent upon environment. The plantlets looking different due to *in vitro* growth may behave as the same plant if taken to the field.

2. The prior art methods require huge space, labour, money and time for screening in the field as the whole lot of regenerant plants are screened.
3. Screening for characters like insect tolerance in the field may lead to environmental degradation through escape of the insect.

The invention claimed in this application relates to a novel and specific procedure targeted to screen, identify and develop insect tolerant plant genotypes or clones involving development of clones through tissue culture as somaclones and simultaneously establishing their molecular distinctiveness through RAPD analysis prior to phenotype evaluation at *in vitro* stage itself. RAPD analysis at this step is critical to be sure that variant plants are selected and not all the plants that arise from tissue culture. This is followed by micropropagation of the identified molecular variants for multiplication and checking for the stability at molecular level through RAPD among clones of larger population which is also critical and unique. The stable variants multiplied by the above method are transferred to individual culture tubes for forced feeding by insects by relating actively feeding larvae or nymphs into each culture tube itself. Then the surviving clones (tolerant to insect attack) are *in-vitro* multiplied and rechecked for insect larval non-preference and then field evaluated under natural or artificial insect infestation conditions to confirm the tolerance to insects.

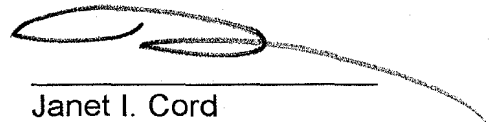
The claims are quite clear and supported by experimentation for its novel method. Field evaluation under natural or artificial insect infestation condition is used to follow up and to validate the insect tolerant clones for performance in the field conditions. The efficiency and high success rate lies with the fact that only those clones are taken for the trials at field level which are confirmed as insect tolerant at the culture stage itself. This is again critical and novel as it reduces a lot of time, space and labour for screening. This invention should be compared as a complete method and not the individual scattered components which can not be just simply added to reach this procedure.

If compared in isolation the individual components of the procedure are: a. Generation of somaclones, b. RAPD analysis, c. screening for insect by feeding with the insect. But the success of this procedure is in the strategy defined in the embodiment with proper sequence of steps and the tact to utilize the outcome of each step for the next.

There is no combination of the references that lead to this method and the advantages associated with the claimed method.

Therefore, it is respectfully requested that this application be passed to issue.

Respectfully submitted

A handwritten signature in dark ink, appearing to read "Janet I. Cord", is written over a horizontal line.

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